

Hitherto, an assisted reproductive technology (ART) has been established, not only in a veterinary field but also in a human sterility treatment. In this ART a spermatozoon and an ovum are fertilized in vitro in a culture system to prepare a fertilized ovum (a zygote). Then the fertilized ovum can be cultured via cleavage, morula and blastocyst stages to a hatching-
blastocyst stage, a late blastula stage wherein azona pellucida is denatured and disappeared, and the fertilized ovum at the stages from cleavage to blastula stage are transplanted in an uterus to obtain a baby.

Please amend the paragraph beginning at page 2, line 7 as follows:

Further, a fertilized ovum (a late blastula) is implanted on an endometrium in vivo and an inner cell mass (an embryoblast) grows to a development stage of early embryo including a gastrula forming process. The early embryo then proceeds to grow to a three-layer embryonic disc. However, at present, no reports of such a growth process in a culture system exists.

Please amend the paragraph beginning at page 2, line 13 as follows:

In the culture systems hitherto presented, even if a fertilized ovum (a late blastula) is cultured continuously, only monolayer cells are proliferated two-dimensionally. Any three-dimensional architecture having an early embryo-like structure, such that a gastrula or a neurula is produced, has yet not been achieved.

Please amend the paragraph beginning at page 2, line 18 as follows:

On one hand, the basic technology of tissue engineering for reconstruction of a tissue from both cultured cells and their scaffold(s) (e.g., a culture carrier(s)) has proceeded eminently for the past 10 years centering around Europe and America. As to organs having relatively simple constructions, a basic reconstruction method has been established [Ferber, D., Science 284, 422-425, (1999)].

Please amend the paragraph beginning at page 2, line 24 as follows:

Previously, many carriers have been developed having various forms, using many

(B) different materials, in order to construct tissues by three-dimensionally assembling cells and extracellular matrix components.

Please amend the paragraph beginning at page 3, line 3 as follows:

The present inventors have established the basic technology of tissue engineering utilizing a mesh network such as cotton gauze as a support (Japanese Patent Application Laid-open No. Hei 7-298876 and Japanese Patent No. 3081130).

Please amend the paragraph beginning at page 3, line 7 as follows:

In Japanese Patent Application Laid-open No. Hei 11-164684, the inventors describe a novel organ engineering method of reconstructing an organ-like construct (an organoid) by subjecting an organ to continuous three-step perfusion to remodel the organ into a culture version organoid without separating the majority of constructive cells in the objective organ.

Please amend the paragraph beginning at page 3, line 13 as follows:

(A) It has been reported that the endometrial epithelial cells reconstructs a uterine gland-like structure by co-culturing human endometrial epithelial cells and stromal cells in a collagen gel [Akoum, A., et al., J. Reprod. Med., 41, 555-561, (1996)].

Please amend the paragraph beginning at page 3, line 18 as follows:

In rabbits, a report exists which cultures endometrial epithelial cells on a matrigel, a reconstituted basement membrane, and thereafter to place a blastocyst just before implantation thereon for co-culturing. Although it is disclosed that cell fusion of a trophoblast (cytotrophoblast) with the endometrial epithelial cells occurred at 48 hours after co-culturing [Tominaga Tosirou, Nihon Sanfujinka Gakkai Zasshi, 48, 591-603, (1996)], there is no description that the cells derived from the blastocyst grow to form a three-dimensional architecture having an early embryo-like structure such that a gastrula or that a neurula is formed.

Please amend the paragraph beginning at page 4, line 4 as follows:

B11 Therefore, at present, no reports exist of a culture carrier or a co-culturing carrier on

which a fertilized ovum of an animal is cultured to induce three-dimensional growth.

Please amend the paragraph beginning at page 4, line 8 as follows:

An object of the invention is to provide a carrier for co-culturing a fertilized ovum of an animal in which behavior of the fertilized ovum of an animal can be easily observed in a culture system and by which adhesion and three-dimensional growth of the fertilized ovum are possible.

Please amend the paragraph beginning at page 4, line 13 as follows:

Another object of the invention is to provide a method of culturing the fertilized ovum of an animal, in which the fertilized ovum of an animal can be grown three-dimensionally by culturing the fertilized ovum of an animal using the co-culturing carrier. This method also permits elucidation of the differences between the three-dimensional growth of the fertilized ovum in an in vitro culture system and the development of the early embryo from the fertilized ovum implanted in vivo, evaluation of teratogenic materials, or grafting of an early embryo developed from the fertilized ovum, et cetera.

Please amend the paragraph beginning at page 4, line 23 as follows:

In order to develop a carrier for co-culturing a fertilized ovum of an animal and a culturing method of a fertilized ovum of an animal in which: a) behavior of the fertilized ovum of an animal can be easily observed in a culture system; and b) adhesion and three-dimensional growth of the fertilized ovum become possible, the inventors have studied diligently. As a result of this study, the inventors have determined that a carrier for co-culturing a fertilized ovum of an animal composed of a cell incorporated type three-dimensionally reconstructed tissue in which cells are beforehand incorporated in a culture carrier makes adhesion and three-dimensional growth of the fertilized ovum possible to complete the present invention.

Please amend the paragraph beginning at page 5, line 11 as follows:

B15 According to the first aspect of the invention is a carrier for co-culturing a fertilized ovum of an animal comprising a cell incorporated type three-dimensionally reconstructed tissue for co-culturing the fertilized ovum of an animal to induce adhesion and three-dimensional growth of the fertilized ovum.

Please amend the paragraph beginning at page 5, line 17 as follows:

B16 According to another aspect of the invention is a method of culturing a fertilized ovum of an animal, wherein any co-culturing carrier as described in the other aspects of the invention is introduced into a culture vessel to culture the fertilized ovum of an animal.

Please amend the paragraph beginning at page 8, line 4 as follows:

B17 In the first embodiment of the present invention is a carrier for co-culturing a fertilized ovum of an animal.

Please amend the paragraph beginning at page 8, line 16 as follows:

B18 As a mammal, there may be mentioned human beings, monkey, bovine, sheep, goat, baboon, pig, dog, guinea pig, rat and mouse etc.

Please amend the paragraph beginning at page 8, line 18 as follows:

B19 The fertilized ovum used in culturing may be any stage of a zygote, cleavage, morula or blastocyst stage, but one grown to a blastocyst stage is preferable as an implantation model.

Please amend the paragraph beginning at page 8, line 21 as follows:

B20 In the culture system according to the first aspect of the invention, any ovum in a life cycle other than a fertilized ovum, namely an ovum cell before fertilization such as an ovum in follicle and an ovulated ovum or a fertilizing ovum, may be used.

Please amend the paragraph beginning at page 9, line 6 as follows:

B21 In addition to the ability that the carrier for co-culturing the fertilized ovum of an animal according to the first aspect of the invention is suitable for adhesion of the fertilized ovum, as well as to support culturing of the fertilized ovum (blastocyte) as hitherto to proliferate

B2) monolayer cells two-dimensionally, but also it can prepare a three-dimensional architecture derived from the fertilized ovum.

Please amend the paragraph beginning at page 10, line 22 as follows:

Such a cell incorporated type three-dimensionally reconstructed tissue is reconstructed from any of cells, tissues or organs derived from animal, and it contains at least one kind of cells as is described in the second aspect of the invention. For example, the cell incorporated type three-dimensionally reconstructed tissue can be obtained by culturing the above-mentioned cells with a culture medium.

Please amend the paragraph beginning at page 11, line 9 as follows:

The cell incorporated type three-dimensionally reconstructed tissue preferably contains an extracellular matrix component and/or a mesh network as is described in the third aspect of the invention. By containing these components, liquid permeability of the culture medium is improved to culture the incorporated cells effectively and to provide tension on the incorporated cells, whereby three-dimensional growth of the fertilized ovum can be conducted in an environment that mimics the living body.

Please amend the paragraph beginning at page 12, line 6 as follows:

B24) Within the context of the present invention, the "mesh network" refers to a fibrous mass having such an opening to form a spatial shape for three-dimensional culturing, and as is described in the eighth aspect of the invention, there may be mentioned natural or synthetic threads and/or woven masses thereof.

Please amend the paragraph beginning at page 13, line 11 as follows:

The mesh network is preferably bioabsorptive according to the ninth aspect of the invention. The term bioabsorption refers to a property to be absorbed and degraded in a living body. Since it can absorb the culture carrier in a living body, it is quite useful for transplantation, etc.

Please amend the paragraph beginning at page 14, line 2 as follows:

In contrast, by containing the mesh network, contraction of the cell incorporated type three-dimensionally reconstructed tissue is inhibited, so that behavior of the fertilized ovum can be preferably observed by means of a phase-contrast microscope.

Please amend the paragraph beginning at page 16, line 6 as follows:

The co-culturing carrier according to the first aspect of the invention, which is composed of the above-mentioned cell incorporated type three-dimensionally reconstructed tissue, can be used for culturing of a fertilized ovum.

Please amend the paragraph beginning at page 17, line 2 as follows:

As the culture medium, any medium suitable for preparing a cell incorporated type three-dimensionally reconstructed tissue may be employed. Such culture medium is changed every one to three day (s). The culturing temperature is 37.0-39.0°C and the culturing period is about 1-60 day (s).

Please amend the paragraph beginning at page 17, line 7 as follows:

When the fertilized ovum is cultured by a culturing method according to the tenth aspect of the invention, the behavior of the fertilized ovum during culturing can be observed by means of a phase-contrast microscope, etc. (see Fig. 6-10). In addition, the cells derived from the fertilized ovum are moved around the fertilized ovum to make three-dimensional growth of the fertilized ovum finally possible (see Fig. 11-14).

Please amend the paragraph beginning at page 17, line 14 as follows:

The first aspect of the invention provides a carrier for co-culturing a fertilized ovum composed of a cell incorporated type three-dimensionally reconstructed tissue, and thus, behavior of the fertilized ovum of an animal in a culture system can be observed easily and adhesion and three-dimensional growth of the fertilized ovum can first become possible.